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SUSCEPTIBILITY OF TEN CLINICAL ISOLATES OF *Pseudomonas aeruginosa* STRAINS AGAINST BENZYLPENICILLIN, GENTAMICIN AND MEROPENEM

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Abstract: About 40% of deaths in people with ventilator-associated pneumonia are caused directly by *P. aeruginosa*. The low susceptibility to antibiotics in *P. aeruginosa* is primarily due to the presence of several multidrug resistance (MDR) efflux pumps encoded in its genome. This study aimed to investigate the susceptibility of ten clinical isolates of *P.aerugina* strains against, Benzylpenicillin, Gentamicin and Meropenem. Broth microdilution was used for assessing minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of each drug in tracheal aspirates samples. Our results indicate good sensitivity of samples to meropenem, and poor susceptibility to the other drugs, and contribute for the local epidemiological monitoring and profiling of drug resistance.
Key Words: Antimicrobial, *Pseudomonas* sp., Susceptibility.

Introduction

Pseudomonas aeruginosa infections are among the most severe that affect patients with cystic fibrosis, sepsis or an immunocompromised status. About 40% of deaths in people with ventilator-associated pneumonia are caused directly by *P. aeruginosa*. After early childhood, a very high percentage of patients with cystic fibrosis suffer from recurrent and finally chronic *P. aeruginosa* pneumonia, a primary cause of lung destruction in these individuals (Paviani et al., 2004; Nakamura et al., 2000; Menezes et al., 2007).

The low susceptibility to antibiotics in *P. aeruginosa* is primarily due to the presence of several multidrug resistance (MDR) efflux pumps encoded in its genome. This represents a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. Other infections caused by *Pseudomonas species* include endocarditic, pneumonia, and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin and musculoskeletal system (Figueiredo et al., 2007; Mesaros et al., 2007).

The availability of antimicrobial drugs to treat infectious diseases has radically improved human and animal well being. Numerous antimicrobials, including most structural classes of antibiotics were discovered during 1920 to 1970, and chemical modification of many of these compounds was performed in order to provide new entities with superior activities. However, a clear decline in antimicrobial drugs investigation marked the pharmaceutical market in the 1990s, and the incorrect use of these drugs in the last decades threatens their future utility (Pires et al., 2009).

Widespread antimicrobial resistance is growing and resulting in impaired treatment of human and animal

diseases. Clinically significant antimicrobial resistance has evolved against virtually every drug deployed. Yet the development of new classes of antimicrobials is far behind the growing need for such drugs. Therefore, the assessment of antimicrobial susceptibility of bacterial strains in clinical settings is important to provide data for a better management on antimicrobial therapy (Poletto and Reis, 2005; Kobayashi et al., 2009).

This work therefore aimed to investigate the susceptibility of clinical isolates of *P.aeruginosa* strains against different antimicrobial drugs. Broth microdilution was used for assessing minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of each drug in tracheal aspirates samples. Our results indicate poor susceptibility of such strains, and contribute for the local epidemiological monitoring and profiling of drug resistance.

Materials and Methods

Bacterial Samples

The clinical isolates were obtained from Microbiology Research Laboratory Collection, University Vale do Rio Doce, and all were from tracheal aspirates of adult patients. Each isolate was tested with the VITEK 2 system (version R04.02, bioMérieux) according to the manufacturer's instructions. A Gram-negative identification card (ID-GNB) was inoculated with a bacterial suspension prepared in 0.9% saline equal to the turbidity of a 0.5 McFarland standard. Discrepant bacterial identifications were resolved by retesting the isolates with the VITEK 2 and reference biochemical tests.

MIC and MBC Assays

Organisms were tested by the CLSI reference method (CLSI, 2010). Microplates contained serial dilutions of antimicrobial drugs at the following concentration ranges: 25 to 0.3 mg/ml for Meropenem (AstraZeneca, UK), 150,000 to 2,343 ui/ml for Benzylpenicillin (Eurofarma, Brazil) , and 400 to 6.25 mg/ml for Gentamicin (Merck, USA).

Plates were inoculated with a bacterial suspension prepared with turbidity of 0.5 McFarland standard, in Mueller-Hinton broth (Difco) and incubated at 35±2 °C overnight. The MIC for each antimicrobial drug tested was the lowest concentration of the drug that inhibited visible growth. To determine the MBC, aliquots of 10 µl were dropped onto the surface of a dried Mueller-Hinton agar plate.

After overnight incubation, the formation of colonies was observed. The MBC for each antimicrobial drug was the lowest concentration that fully inhibited bacterial growth in agar.

Results and Discussion

Table 1 shows the susceptibility of *P. aeruginosa* samples to Meropenem. All strains presented MCB of 0.7 mg/ml. Susceptibility data of *P. aeruginosa* regarding this drug is scarce. White et al. (1996) conducted a study in which the susceptibility of *P. aeruginosa* ATCC 27853 to meropenem was assessed, and obtained an MBC of 0.5 mg/ml. It should be considered that this is one of the first studies about the profile of susceptibility of *Pseudomonas* samples to meropenem, and that the work was performed only with an ATCC strain. Another study performed by Nakamura et al. (2000) have described a meropenem MIC of 0.39 mg/ml to 15 strains of *P. aeruginosa*.

	Concentration of Meropenem (mg/mL)						
Strain	25	12.5	6.2	3.1	1.5	0.7	0.3
P.aeruginosa 1	-	-	-	-	-	-	+
P.aeruginosa 2	-	-	-	-	-	-	+
P.aeruginosa 3	-	-	-	-	-	-	+
P.aeruginosa 4	-	-	-	-	-	-	+
P.aeruginosa 5	-	-	-	-	-	-	+
P.aeruginosa 6	-	-	-	-	-	-	+
P.aeruginosa 7	-	-	-	-	-	-	+
P.aeruginosa 8	-	-	-	-	-	-	+
P. aeruginosa 9	-	-	-	-	-	-	+
P. aeruginosa 10	-	-	-	-	-	-	+

Table 1: Susceptibility profile of samples to meropenem.

Table 2 presents the susceptibility of the samples to Benzylpenicillin. All strains presented MBC of 150.000 IU/ml. A reduced spectrum of action has been described for this drug, and due to the increasing emergence of β-lactamase-producing strains, it is increasingly narrowing, and its use has been reported as a clinical problem for a long time (Suginaka et al., 1975).

	Concentration of Benzylpenicillin (IU/ml)						
Strain	150.000	75.000	37.500	18.750	9.375	4.687	2.343
P.aeruginosa 1	-	+	+	+	+	+	+
P.aeruginosa 2	-	+	+	+	+	+	+
P.aeruginosa 3	-	+	+	+	+	+	+
P.aeruginosa 4	-	+	+	+	+	+	+
P.aeruginosa 5	-	+	+	+	+	+	+
P.aeruginosa 6	-	+	+	+	+	+	+
P.aeruginosa 7	-	+	+	+	+	+	+
P.aeruginosa 8	-	+	+	+	+	+	+
P. aeruginosa 9	-	+	+	+	+	+	+
P. aeruginosa 10	-	+	+	+	+	+	+

Table 2: Susceptibility profile of samples to Benzylpenicillin.

In Table 3 it is shown the susceptibility of samples to gentamicin. MBC ranged from 400 to 6.25 µg/mL, however, the concentration of 200 µg/mL was the lower effective dose for all strains, and therefore, considered the MBC. Sader et al. (2001) observed a sensitivity of 56.3% to gentamicin from samples of *P. aeruginosa*. Paviani et al. (2004), among the 72 isolates of *P. aeruginosa* inpatient units, found susceptibility of 38% for gentamicin. Among the 49 isolates of *P. aeruginosa* from intensive treatment center, the sensitivity was of 33%. These results are encouraging; however, they contrast with our data, where the reports were of varied resistance.

	Concentration of Gentamicin (µg/mL)						
Strain	400	200	100	50	25	12,5	6,25
P.aeruginosa 1	-	-	-	-	-	-	+
P.aeruginosa 2	-	-	-	-	-	+	+
P.aeruginosa 3	-	-	-	-	-	+	+
P.aeruginosa 4	-	-	-	+	+	+	+
P.aeruginosa 5	-	-	-	+	+	+	+
P.aeruginosa 6	-	-	+	+	+	+	+
P.aeruginosa 7	-	-	+	+	+	+	+
P.aeruginosa 8	-	-	-	-	-	-	+
P. aeruginosa 9	-	-	-	-	-	-	+
P. aeruginosa 10	-	-	-	-	-	+	+

Table 3: Susceptibility profile of samples to Gentamicin

There are few scientific studies that describe the MBC of *P. aeruginosa* and of the antimicrobials tested. Whereas this study represents the behavior of the patient antimicrobial immune suppressed, it becomes of great importance, since the MBC is the lowest concentration of an antimicrobial agent capable of reducing the microbial count on 99.9%. Determination of MBC of antibiotic for an infecting organism is now more frequently requested of clinical microbiology laboratories because infections in immunocompromised and other patients may require treatment with bactericidal rather than bacteriostatic levels (Pires et al., 2009).

Conclusion

Appropriate use of antimicrobials in health care continues to be a challenge. Meropenem and gentamicin were the most effective drugs against the clinical isolates of *P. aeruginosa* in this study. It is important to continue susceptibility testing of clinical bacterial isolates because in order to identify possible resistant strains and to provide the clinician valuable information that can be translated into positive clinical outcomes at the bedside. Despite this study has the limitation regarding the samples being related to a single inner-city, our data is still important for local epidemiologic diagnosis, which are scarce.

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